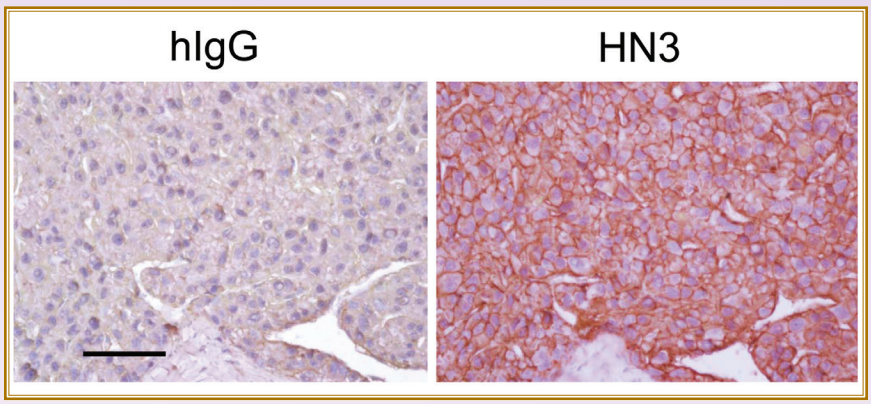
Novel Antibody Targets Glypican-3 in Liver Cancer

New treatments for liver cancer are greatly needed, because hepatocellular carcinoma (HCC), the most common type of liver cancer, is particularly insensitive to chemotherapy. Surgery is standard for HCCs caught early, but only a third of cases are identified at this stage. Antibody therapy offers a potential alternative for later-stage tumors.

An attractive antibody target is glypican-3 (GPC3), a cell surface associated-protein that is overexpressed on HCC cells but not on normal liver tissue. While its exact function is not well understood, GPC3 seems to help regulate cell growth. Mitchell Ho, Ph.D., in CCR's Laboratory of Molecular Biology, and his colleagues decided to investigate the role of GPC3 in HCC and develop GPC3-specific antibodies. Their findings were recently published in the *Proceedings of the National Academy of Science*.

To identify antibody domains targeting GPC3, the investigators screened a phage library of heavy chain variable domains, which can interact with small pockets or other regions that full-length antibodies cannot, for their GPC3-binding ability. The library was previously described by Dimiter Dimitrov, Ph.D., in CCR's Nanobiology Program. After four rounds of phage panning, Mingqian Feng, Ph.D., a Postdoctoral Fellow in the Ho lab, identified four interacting domains. The HN3 domain had the strongest association and was used for subsequent studies.

The researchers cloned the HN3 domain into a vector that fused it with the constant region of a human antibody. Only cells that expressed GPC3 bound to the antibody. To see what effect HN3 had on cell growth, the researchers treated four HCC lines with the antibody. HN3 significantly reduced growth in three of the four lines at 0.1μM and reduced the growth of the fourth at 1.0μM. A cell line that normally lacks GPC3 was not affected by HN3.



(Image: M. Ho, CCR)

CCR scientists generated a new human single-domain antibody, HN3, that recognizes GPC3 for liver cancer therapy. The human antibody strongly bound HCC cells (right) and inhibited tumor cell proliferation via inactivating Hippo/yap signaling. Furthermore, HN3 significantly inhibited growth of HCC tumor xenografts in mice. hIgG is pooled human IgG used as control. Scale bar, 100 μm.

To understand how HN3 caused growth arrest, the scientists examined several pro-growth signaling pathways. HN3 treatment decreased phospho-Erk and phospho-Akt levels in four HCC lines. Levels of phospho-yap, an inactive form of this Hippo pathway member, increased in three of the lines. Expression of cyclin D1, a yap target, decreased in all four. Because Erk and Akt can affect Hippo signaling, these results suggested that yap facilitates HN3-mediated cell cycle arrest.

To test this idea, the researchers over-expressed constitutively-active yap or knocked down yap in an HCC line and evaluated HN3-induced growth arrest. Cells over-expressing active yap proliferated more and were insensitive to HN3. Conversely, cells lacking yap had much lower growth rates. Adding HN3 antibody had no effect. These studies indicate that yap is an important target of HN3 and GPC3 signaling.

Finally, the researchers treated mice bearing HCC tumors with HN3 or a control twice a week. HN3 treatment significantly reduced the size of tumors from two HCC lines. The scientists also detected increases in phospho-yap and decreases in cyclin D1 and phospho-Erk in treated tumors. The investigators noted no antibody-related toxicities and suggested that HN3 should undergo further testing as a potential therapeutic for liver cancer.

To learn more about Dr. Ho's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?name=ho.